space. METHODS/STUDY POPULATION: Methods: We prospectively recruited 39 patients undergoing prostatectomy for this institutional review board (IRB) approved study. Patients underwent MP-MRI before prostatectomy on a 3T field strength MRI scanner (General Electric, Waukesha, WI, USA) using an endorectal coil. MP-MRI included field-of-view optimized and constrained undistorted single shot (FOCUS) diffusion weighted imaging with 10 *b*-values ($b = 0$, 10, 25, 50, 80, 100, 200, 500, 1000, and 2000), dynamic contrast enhanced imaging, and T2-weighted imaging. T2 weighted images were intensity normalized and apparent diffusion coefficient maps were calculated. The dynamic contrast enhanced data was used to calculate the percent change in signal intensity before and after contrast injection. All images were aligned to the T2 weighted image. Robotic prostatectomy was performed 2 weeks after image acquisition. Prostate samples were sliced using a 3D printed slicing jig matching the slice profile of the T2 weighted image. Whole mount samples at 10 µm thickness were taken, hematoxylin and eosin stained, digitized, and annotated by a board certified pathologist. A total of 210 slides were included in this study. Lumen and epithelium were automatically segmented using a custom algorithm written in MATLAB. The algorithm was validated by comparing manual to automatic segmentation on 18 samples. Slides were aligned with the T2 weighted image using a nonlinear control point warping technique. Lumen and epithelium density and the expert annotation were subsequently transformed into MRI space. Co-registration was validated by applying a known warp to tumor masks noted by the pathologist and control point warping the whole mount slide to match the transform. Overlap was measured using a DICE coefficient. A learning curve was generated to determine the optimal number of patients to train the algorithm on. A PLS algorithm was trained on 150 random permutations of patients incrementing from 1 to 29 patients. Slides were stratified such that all slides from a single patient were in the same cohort. Three cohorts were generated, with tumor burden balanced across all cohort. A PLS algorithm was trained on 2 independent training sets (cohorts 1 and 2) and applied to cohort 3. The input vector consisted of MRI values and the target variable was lumen and epithelium density. The algorithm was trained lesion-wise. Trained PiCT models were applied to the test cohort voxel-wise to generate 2 new image contrasts. Mean lesion values were compared between high grade, low grade, and healthy tissue using an ANOVA. An ROC analysis was performed lesion-wise on the test set. RESULTS/ANTICIPATED RESULTS: Results: The segmentation accuracy validation revealed $R = 0.99$ and $R = 0.72$ ($p < 0.001$) for lumen and epithelium, respectively. The co-registration accuracy revealed a 94.5% overlap. The learning curve stabilized at 10 patients with a root mean square error of 0.14, thus the size of the 2 independent training cohorts was set to 10, leaving 19 for the test cohort. DISCUSSION/SIGNIFICANCE OF IMPACT: We present a technique for combining radiology and pathology with machine learning for generating predictive cytological topography (PiCT) maps of cellularity and lumen density prostate. The voxel-wise approach to mapping cellular features generates 2 new interpretable image contrasts, which can potentially increase confidence in diagnosis or guide biopsy and radiation treatment.

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PRMT5 is a master epigenetic regulator to promote repair of radiation-induced DNA damage

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OBJECTIVES/SPECIFIC AIMS: We recently reported that PRMT5 epigenetically activates androgen receptor (AR) in prostate cancer cells. Because targeting AR signaling through androgen deprivation therapy is clinically used as a radiosensitization approach to treat high-risk prostate cancer, our finding raised an exciting possibility that targeting PRMT5 may improve RT for prostate cancer patients. Contrary to our expectation, targeting PRMT5 sensitized both AR expressing and AR negative (AR−) prostate cancer cell lines to radiation. The goal of our study was therefore to determine the role of PRMT5 in repair of IR-induced DSBs and to translate these findings to improving radiation therapy for cancer patients in general (not just prostate cancer patients). METHODS/STUDY POPULATION: The majority of experiments were basic science experiments analyzing PRMT5's role in the DNA damage response in normal and cancer cell lines. For example, to extend our findings and determine if PRMT5's role in DSB repair is conserved across multiple cell types, we performed similar experiments in AR− prostate cancer cells, luminal breast cancer cells, glioblastoma cells, and human embryonic kidney cells. To determine the clinical significance of our finding, we also analyzed mRNA expression of PRMT5, AR, and both PRMT5 and AR target genes involved in DSB repair across 43 clinical cancer data sets. RESULTS/ANTICIPATED RESULTS: (1) Targeting PRMT5 sensitizes prostate cancer cells to IR in an AR-independent manner, (2) PRMT5 regulates the repair of IR-induced DSBs in an AR-independent manner, (3) RNA-seq analysis reveals that PRMT5 likely regulates genes involved in the DNA damage response, (4) PRMT5 activates expression of several genes in the DDR including those involved in DSB repair, (5) PRMT5 functions as an epigenetic activator of genes involved in DDR, (6) PRMT5 is required for NHEJ, HR, and G2-Arrest upon IR treatment, (7) Upregulation of PRMT5 correlates with formation and repair of IR-induced DSBs, (8) PRMT5's role in repair of IR-induced DSBs is conserved in several normal and cancer cell types, and (9) PRMT5 expression correlates with expression of DSB repair proteins in clinical cancer samples. DISCUSSION/SIGNIFICANCE OF IMPACT: In summary, we provide evidence that PRMT5 is a master epigenetic regulator of IR-induced DSB repair through epigenetic activation of multiple target genes involved both HR and NHEJ as well as G2 arrest. Interestingly, the majority of genes regulated by PRMT5 are well-characterized, "core repair proteins" involved in HR (RAD51, BRCA1, BRCA2, RAD51D, and RAD51AP1), NHEJ (NHEJ1, Ku80, XRCC4, and DNAPKcs), and G2 arrest (Cdk1, CDC25C, CCNB2, and WEE1), which may explain why PRMT5 is essential to repair IR-induced DSBs in several cell lines. Although AR may also regulate DSB repair via both HR and NHEJ, several pieces of evidence in our study suggest that PRMT5 also regulates DSB repair independent of AR. First, PRMT5 targeting sensitizes both AR + and AR − prostate cancer cells to IR. Second, exogenous expression of AR only partially rescues the impairment of IR-induced DSB repair by PRMT5 knockdown. Third, PRMT5 knockdown increases IR-induced DSB in AR − DU145 cells and several other cancer cell lines and normal cells. Fourth, PRMT5 expression correlates positively with the expression of its target genes in multiple human cancer tissues. During preparation of this project, Braun *et al.* reported that PRMT5 post-translationally regulates the splicing out of detained-introns (DI)s of genes to modulate gene expression. However, analysis of their data showed that the majority of DEGs we identified either do not contain DIs or DI splicing was not affected by targeting PRMT5. In addition, Clarke *et al.* reported that PRMT5 participates in the DSB repair choice process and promotes HR through methylation of RUVBL1. It is therefore likely that PRMT5 regulates repair of IR-induced DSB via multiple mechanisms. As PRMT5 is overexpressed in many human cancers and its overexpression correlates with poor prognosis, our findings suggest that increased DSB repair by PRMT5 overexpression in these human cancers may confer survival advantages particularly following DNA damaging treatment. Because targeting DSB repair has been proven to be a valid therapeutic approach for cancer treatment, our findings here also suggest that PRMT5 targeting may be explored as a monotherapy or in combination therapy with RT or chemotherapy for cancer treatment.

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Protein production as an early pharmacodynamics biomarker for RNA-targeting therapies

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OBJECTIVES/SPECIFIC AIMS: We aimed to develop an assay to measure new protein synthesis after Antisense Oligonucleotide treatment, which we hypothesized to be the earliest biochemical identification of RNA-targeting therapy efficacy. METHODS/STUDY POPULATION: We treated 2 transgenic animal models expressing proteins implicated in neurodegenerative disease: human tau protein (hTau) and human superoxide dismutase 1 (hSOD1), with ASO against these mRNA transcripts. Animals received isotope-labeled 13C6-Leucine via drinking water to label newly synthesized proteins. We assayed target protein synthesis and concentration after ASO treatment to determine the earliest identification of ASO target engagement. RESULTS/ANTICIPATED RESULTS: hTau ASO treatment in transgenic mice lowered hTau protein concentration 23 days post-treatment in cortex (95% CI: 0.05%–64.0% reduction). In the same tissue, we observed lowering of hTau protein synthesis as early as 13 days (95% CI: 29.4%–123%). In hSOD1 transgenic rats, we observed lowering of 13C6-leucine-labeled hSOD1 in the cerebrospinal fluid 30 days after ASO treatment compared with inactive ASO control (95% CI: 12.0%–48.4%). DISCUSSION/SIGNIFICANCE OF IMPACT: In progressive neurodegenerative diseases, it is crucial to develop measurements that identify treatment efficacy early to improve patient outcomes. These data support the use of stable isotope labeling of amino acids to measure new protein synthesis as an early pharmacodynamics measurement for therapies that target RNA and inhibit the translation of proteins.

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Proteomics in the early diagnosis of metabolic syndrome in a Hispanic pre-teen cohort

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OBJECTIVES/SPECIFIC AIMS: The objective of the present study is to determine if decreased adiponectin and increased leptin levels are associated